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REVIEW

Neural functions of long noncoding RNAs in *Drosophila*

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Abstract Long noncoding RNA (lncRNA) is an emerging category of transcript, and comprises the majority of the transcriptome of various complex organisms. The biological functions of only a handful of lncRNAs have been investigated in detail, showing involvement in a wide range of biological processes through different functional paradigms. However, most lncRNAs remain to be identified. Many lncRNAs are predicted to function, often preferentially, in the nervous system, potentially playing roles in mediating neural functions such as development, behavior, and cognition. To examine the biological significance and potential mechanisms of the remaining unknown neural lncRNAs, certain tractable model organisms, such as *Drosophila*, can provide advantages including the use of numerous genetic tools. Herein, we summarize recent progress on the in vivo or potential functions of *Drosophila* lncRNAs, in particular, behavior and development-related lncRNAs.

Keywords Behavior · Development · Long noncoding RNA · *Drosophila*

Introduction

Long noncoding RNAs (lncRNAs) are RNA transcripts with no protein-coding capacity, which are longer than 200 nucleotides and lack appreciable opening reading frames (Ng et al. 2013). Similar to mRNA, some lncRNAs are transcribed by RNA polymerase II and processed via 5′ end-capping, 3′ end-polyadenylation, and alternative splicing (Kapranov et al. 2007; Qureshi et al. 2010). lncRNAs can be classified according to their genomic organization. Sense or anti-sense lncRNAs share overlapping regions with adjacent protein-coding genes on the same or opposite strand, respectively (Martens et al. 2004; Feng et al. 2006). Bidirectional lncRNAs originates their expression and the neighboring coding transcript on the opposite strand in close genomic vicinity (Faedo et al. 2004), while intronic lncRNAs derive from introns of splicing coding transcripts (Heo and Sung 2011) and intergenic lncRNAs are located within the genomic interval between two coding genes (Collier et al. 2012). As for functional paradigms, lncRNAs can regulate gene expression at the level of transcription, for example the yeast lncRNA, *SRG1*, disrupts the expression of the downstream *SER3* gene (Martens et al. 2004) (Fig. 1a), and *CRG*, a *Drosophila* lncRNA, positively regulates the expression of upstream *CASK* by recruiting the transcription initiation complex to the *CASK* gene promoter (Li et al. 2012) (Fig. 1b). lncRNAs can also induce chromatin modifications (e.g., Hox antisense intergenic RNA (*HOTAIR*) transcribed from the *HoxC* locus, recruits the Polycomb chromatin remodeling complex (PRC2), resulting in histone methylation and gene silencing in the distal *HoxD* locus) (Rinn et al. 2007) (Fig. 1c), and alter subcellular trafficking (e.g., *NRON* regulates the localization of the transcription factor, NFAT, by interacting with nuclear transport proteins) (Willingham et al. 2005) (Fig. 1d).

Similar to vertebrates, the transcriptomes of invertebrates such as *Drosophila*, consist of a large number of lncRNAs (Kapranov et al. 2007; Guttman and Rinn 2012; Rinn and Chang 2012; Young et al. 2012). To date, only a very small portion of known lncRNAs have been thoroughly characterized, but with diverse biological functions shown for those described (Martens et al. 2004; Feng

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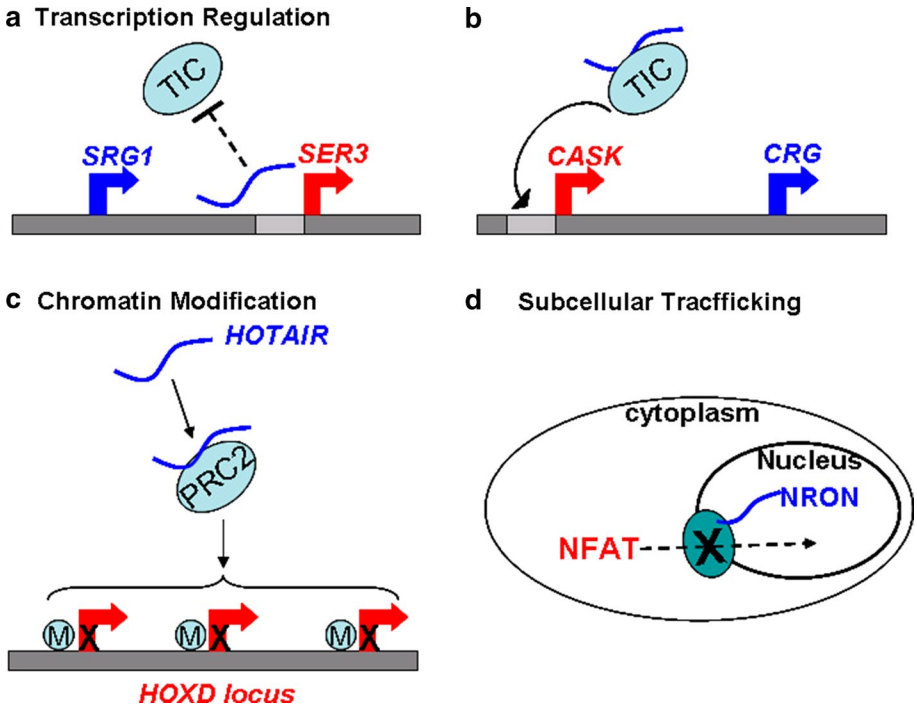

Fig. 1 Functional paradigms of lncRNAs. **a** Transcriptional interference. Transcription of upstream *SRG1* via the *SER3* promoter disturbs *SER3* expression. **b** Promoter enhancement. *CRG* recruits RNA polymerase II to the *CASK* promoter. **c** Epigenetic repression. *HOTAIR* interacts with PRC2 resulting in histone methylation of the *HoxD* locus. **d** Transport of transcription factors. *NRON* binds to a nuclear transport protein to inhibit nuclear translocation of NFAT. *Blue* lncRNAs, *red* protein-coding gene, *pale gray* promoter elements

et al. 2006; Martianov et al. 2007; Hirota et al. 2008; Zhao et al. 2008). Although currently little is known about the *in vivo* functions of most lncRNAs, tissue-specific expression patterns have been demonstrated, with especially high expression observed in the nervous system of both mammals and fruit flies (Inagaki et al. 2005; Muotri and Gage 2006; Mehler and Mattick 2006, 2007; Mercer et al. 2008; Ponjavic et al. 2009). Neural abundance and specificity of lncRNAs suggests they may have crucial effects on neural development, cognitive activity, and neurodegenerative processes. This review discusses recent findings on the roles of lncRNAs, particularly in mediating behavior or development, using *Drosophila* as a genetic model to gain insight into the pathogenesis of neurological diseases with movement or cognitive dysfunction.

Bioinformatic and expression analyses indicate abundant neural-related lncRNAs in *Drosophila*

Recently, a set of 1,119 putative, long intergenic noncoding RNAs (lincRNAs) were identified using modENCODE whole transcriptome (RNA-seq) data from *D. melanogaster* (Young et al. 2012). All the identified lincRNAs exhibit dynamic expression profiles throughout the flies' lifecycle. To determine potential biological functions, genomic loci were examined and found to be significantly enriched in the proximity of development-related protein-coding genes mediating nervous system development, imaginal disc-derived wing morphogenesis, and sensory organ or ventral cord development. Moreover, high correlation of expression levels between lincRNAs and adjacent development-involved protein-coding genes suggests the lincRNAs likely play roles in regulating developmental processes, particularly those of the nervous system (Young et al. 2012). To date, this study provides the best lincRNA candidates for further experimental investigation on contributions to neural developmental processes.

Another *in silico* screening study identified 136 unannotated mRNA-like noncoding RNAs (ncRNAs) in *Drosophila*, and used *in situ* hybridization to determine expression at the embryonic stage. One quarter of the transcripts were detected during embryogenesis, and most exhibited tissue-specific expression patterns, particularly high in the central and peripheral nervous systems (Inagaki et al. 2005). This suggests that mRNA-like ncRNAs play important roles in organogenesis and cell differentiation during *Drosophila* development.

Overall, these studies predict that vertebrate and invertebrate genomes hold large numbers of lncRNA loci with strong neural correlations. Thus, *Drosophila* genetics will allow investigation of lncRNA neural functions *in vivo*, and in particular, effects on cognition or behavior and potential molecular mechanisms.

The lncRNA *yar* affects *Drosophila* sleep behavior

Drosophila yellow-achaete intergenic RNA (*yar*) is an intergenic lncRNA. Upstream of *yar* is the *yellow* gene

(*y*), encoding a secreted protein required for cuticle coloration and male sexual behavior (Nash and Yarkin 1974; Biessmann 1985; Chia et al. 1986; Geyer et al. 1986; Geyer and Corces 1987; Drapeau et al. 2003), while downstream is the *achaete* gene (*ac*), which encodes one of the four related basic HLH transcription factors of the *achaete-scute* complex (*AS-C*), responsible for proper development of the central and peripheral nervous systems (Modolell and Campuzano 1998; Gibert and Simpson 2003; Negre and Simpson 2009). Because gene order in eukaryotic chromosomes is nonrandom and *yar* is located in a neural gene cluster, by inference from neighboring gene functions, it suggests that *yar* may have a neural function (Soshnev et al. 2008). In addition, open reading frame (ORF) analysis demonstrated that *yar* is an lncRNA gene. Thereafter, two general fly behaviors (geotactic ability and sleep) were investigated in adult flies, showing that *yar* null mutants exhibit normal locomotor behavior, but have shortened night-time sleep bouts within a normal circadian sleep-wake cycle and diminished levels of sleep rebound following deprivation. The two defective night-time sleep and sleep rebound phenotypes can be rescued by a *yar* transgene, demonstrating that *yar* is required for sleep regulation (Soshnev et al. 2011). During mid-embryogenesis, an ubiquitous *yar* RNA expression pattern was also shown. Complementary and supporting evidence for behavioral regulation by *yar* will benefit from future expression analysis on *yar* RNA at the adult stage. As for the potential molecular mechanism of *yar* involvement in sleep regulation, first, loss of *yar* does not affect transcription of the two adjacent genes, *y,* and *ac*, therefore excludes these genes as *yar* candidate target genes. Second, as *yar* is a cytoplasmic RNA, it suggests that the regulatory effects of *yar* likely depend upon stabilization or translational regulation of target RNAs. This study provides an example of an lncRNA mediating *Drosophila* sleep behavior, which will aid investigation of vertebrate lncRNAs with similar functions and potentially identify the molecular basis of sleep regulation.

The lncRNA *CRG* regulates *Drosophila* locomotor behavior

Many lncRNAs show spatial- and temporal-specific expression patterns within the central nervous system, suggesting they play important roles in cellular processes, neural development, and cognitive and behavioral processes. In previous studies, we identified lncRNAs using bioinformatic and in vitro translation assays (Li et al. 2012, 2014), and detected *CASK* regulatory gene (*CRG*), a novel behavior-related lncRNA (Li et al. 2012). *CRG* has a restricted expression pattern within the central nervous system at the embryonic, third instar larval, and adult stages. In the *Drosophila* genome, *CRG* is located downstream of a behavior-related coding gene, *CASK* (Martin and Ollo 1996; Slawson et al. 2011), with an overlapping region between the *CRG* 5′ end and *CASK* 3′ UTR region. Because of this neural-specific expression pattern and location adjacent to a behavior-related coding gene, we investigated fly behavior in *CRG* null mutants and found they have defective locomotor behavior, exhibited as a shorter tracing length and lower climbing index using Buridan's paradigm and climbing assay, respectively. The two locomotor defects were restored by *CRG* overexpression, confirming *CRG* involvement in locomotor behavioral regulation. As for the *CRG* target gene responsible for behavioral regulation, *CASK* RNA and protein levels were down-regulated in *CRG* null mutants and reversed by *CRG* restoration. In addition, the two defective behaviors were also rescued by *CASK* overexpression in a *CRG* null mutant background, suggesting that *CRG-CASK* signaling mediates both locomotor behaviors. At the molecular level, *CRG* was shown to recruit RNA polymerase II to the *CASK* promoter and enhance *CASK* expression. Furthermore, *CRG* interactions between both RNA polymerase II and *CRG* functional domains were identified. Our study described *CRG*, a novel neural-specific lncRNA, involved in *Drosophila* locomotor behavior via transcriptional regulation of adjacent protein-coding genes, and demonstrated another lncRNA functional mode, thereby further enriching their biological significance. We used the fruit fly, a genetic model organism, to identify a novel behavior-related lncRNA and clarify underlying molecular mechanisms. Our approach may provide insight into the pathogenesis of neurological diseases associated with movement disorders.

The lncRNA *iab-8* affects mating behavior in *Drosophila*

The *Drosophila* bithorax complex determines the posterior thorax and each abdominal segment of the fly, by regulating expression of three homeotic genes: *Ultrabithorax* (*Ubx*), *abdominal A* (*abd-A*), and *Abdominal B* (*Abd-B*) (Lewis 1978). *Drosophila* homeotic gene clusters contain many lncRNAs, and including *iab-8* (Lipshitz et al. 1987; Cumberledge et al. 1990; Bae et al. 2002; Stark et al. 2008; Tyler et al. 2008). The 92-kb-long *iab-8* transcript is encoded in the intergenic region between the homeotic *abd-A* and *Abd-B* genes. lncRNA *iab-8* is expressed in neural cells of the eighth abdominal segment from embryonic stage 14, and represses *abd-A*. *Abd-A* repression by *iab-8* involves two mechanisms, one uses a microRNA (miR-iab-8) imbedded within an intron of the *iab-8* ncRNA, while the other is mediated by transcriptional interference when RNA polymerase reaches the 3′ end of the *iab-8* ncRNA overlapping

with the *abd-A* promoter (Gummalla et al. 2012). Knocking down *iab-8* expression induces male and female sterility. This sterility does not derive from a problem with gametogenesis, gonads, or the external genitalia, but originates from a behavioral phenotype, and specifically, the male abdomen fails to bend and thereby prevents copulation with female flies. In female flies, eggs cannot pass through the oviduct, possibly because of a peristaltic wave disorder (Gummalla et al. 2012).

The lncRNA *bft* contributes to bristle morphogenesis

Sensory organ formation is regulated by both lineage and selector genes. Lineage genes such as *tramtrack* (*ttk*), are expressed in external sensory and internal chordotonal organs, and direct asymmetric division of sensory organ precursors (Uemura et al. 1989; Rhyu et al. 1994; Guo et al. 1995). Selector genes such as *cut,* are expressed in external sensory organ precursors and their progeny, and specify organ identity (Bodmer et al. 1987; Blochlinger et al. 1988, 1990, 1991). For appropriate organogenesis of the sensillum structures, organ identity and lineage information must be integrated within individual cells of a sensory organ. The *Drosophila* peripheral nervous system lncRNA *bereft* (*bft*), which is expressed in external sensory organ support cells, participates in this integration. *Bft* acts downstream of *cut* and *ttk* to implement correct morphogenesis of cuticular structure forming support cells, and in particular those of the interommatidial bristles of the eye (Hardiman et al. 2002).

Hsrω is a stress-responsive *Drosophila* lncRNA

In *D. melanogaster*, the *heat shock RNA omega* (*hsrω*) lncRNA is one of the most active genes after heat exposure (Lakhotia 2003). *Hsrω* loci in different *Drosophila* species share a common organization with two exons, one intron, and a long stretch of tandem repeats at the 3′ end of the gene. The *hsrω* gene produces three splicing transcripts (hsrω-pre-c, hsrω-c, and hsrω-n), with hsrω-c located in the cytoplasm and the other two restricted to the nucleus (Garbe et al. 1986). Multiple *hsrω* transcripts are expressed in nearly all cells from the embryonic to adult stages, in a developmentally regulated pattern essential for normal development and stressful conditions, such as heat shock (Bendena et al. 1991; Mutsuddi and Lakhotia 1995; Lakhotia et al. 2001). Of the *hsrω* transcripts, the large (>10 kb) nuclear-restricted hsrω-n transcript is responsible for spatial restoration of key regulatory factors (e.g., hnRNPs, HP1, and RNA polymerase II) to their pre-stress nuclear targets in cells recovering from thermal stress. Failure of correct relocation to pre-stress chromosome sites induces restoration failure for normal developmental gene activity and finally delayed organismal death (Lakhotia et al. 2012). Thus, this study provides insight into regulation of cellular reprogramming events at the beginning of stress recovery and highlights the importance of the hsrω-n transcript for organismal survival.

The *Drosophila* lncRNA *bxd* regulates *Ubx* transcription

Drosophila Hox genes regulate anterior–posterior patterning, and misexpression can cause homeotic transformations (Lewis 1978). In *D. melanogaster*, Hox gene intergenic regions produce many long ncRNAs that may regulate Hox gene transcription, e.g., *bithoraxoid* (*bxd*). There are several studies concerning different regulatory modes of *bxd* on *Ubx* transcription. An embryonic study found a non-overlapping pattern for anterior *bxd* and posterior *Ubx*, with *bxd* repressing *Ubx* expression in *cis* through a transcription-dependent mechanism. Further, alternative association of trithorax acetylation complex (TAC1) with either *bxd* or *Ubx* induces transcription and repression of the associated and non-associated one, respectively. Thus, the mosaic pattern of *Ubx* expression induced by TAC1 promotes elongation of *bxd* RNA and inhibits *Ubx* expression (Petruk et al. 2006). *Ubx* RNA de-repression was also shown by deleting the *bxd* ncRNA promoter (Sipos et al. 2007). In another embryonic study, an inverted *bxd* ncRNA promoter within the bithorax complex, produced nonsense *bxd* RNA and induced three additional stripes of *Ubx* RNA expression in blastoderm embryos. Therefore, *bxd* RNA delays the appearance of the posterior three stripes of *Ubx* RNA, but does not affect fly development (Pease et al. 2013). In summary, *Ubx* transcription is regulated by *bxd* RNA in specific locations and developmental stages.

Conclusions

LncRNA transcription is widespread, not only in the mammalian genome but also in invertebrates, such as *Drosophila*. However, there is weak conservation between lncRNA sequences of different species, suggesting they are frequently acted upon by positive selection (Hyashizaki 2004; Pang et al. 2006; Ponjavic et al. 2007). Many lncRNAs have been predicted or demonstrated experimentally to have regulatory effects on neural functions, which is of importance, as dysfunction of this regulatory network may induce neurological disorders. Thus, using powerful genetic tools in *Drosophila* may provide valuable insight into in vivo lncRNA regulatory effects on development,

behavior, and cognition. This enables the molecular basis or neural region underlying phenotypic alterations elicited by novel lncRNAs to be determined, thereby further enriching their biological significance, particularly in terms of neural function.

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References

Bae E, Calhoun VC, Levine M, Lewis EB, Drewell RA (2002) Characterization of the intergenic RNA profile at abdominal-A and -B in the *Drosophila* bithorax complex. Proc Natl Acad Sci USA 99:16847–16852

Bendena WG, Ayme-Southgate A, Garbe JC, Pardue ML (1991) Expression of heat-shock locus hsr-omega in nonstressed cells during development in *Drosophila melanogaster*. Dev Biol 144:65–77

Biessmann H (1985) Molecular analysis of the *yellow* gene (*y*) region of *Drosophila melanogaster*. Proc Natl Acad Sci USA 82:7369–7373

Blochlinger K, Bodmer R, Jack J, Jan LY, Jan YN (1988) Primary structure and expression of a product from *cut*, a locus involved in specifying sensory organ identity in *Drosophila*. Nature 333:629–635

Blochlinger K, Bodmer R, Jan LY, Jan YN (1990) Patterns of expression of *cut*, a protein required for external sensory organ development in wild-type and *cut* mutant *Drosophila* embryos. Genes Dev 4:1322–1331

Blochlinger K, Jan LY, Jan YN (1991) Transformation of sensory organ identity by ectopic expression of Cut in *Drosophila*. Genes Dev 5:1124–1135

Bodmer R, Barbel S, Sheperd S, Jack JW, Jan LY, Jan YN (1987) Transformation of sensory organs by mutations of the *cut* locus of *D. melanogaster*. Cell 51:293–307

Chia W, Howes G, Martin M, Meng YB, Moses K, Tsubota S (1986) Molecular analysis of the *yellow* locus of *Drosophila*. EMBO J 5:3597–3605

Collier SP, Collins PL, Williams CL, Boothby MR, Aune TM (2012) Cutting edge: influence of Tmevpg1, a long intergenic noncoding RNA, on the expression of Ifng by Th1 cells. J Immunol 189:2084–2088

Cumberledge S, Zaratzian A, Sakonju S (1990) Characterization of two RNAs transcribed from the cis-regulatory region of the abd-A domain within the *Drosophila* bithorax complex. Proc Natl Acad Sci USA 87:3259–3263

Drapeau MD, Radovic A, Wittkopp PJ, Long AD (2003) A gene necessary for normal male courtship, *yellow*, acts downstream of fruitless in the *Drosophila melanogaster* larval brain. J Neurobiol 55:53–72

Faedo A, Quinn JC, Stoney P, Long JE, Dye C, Zollo M, Rubenstein JL, Price DJ, Bulfone A (2004) Identification and characterization of a novel transcript down-regulated in Dlx1/Dlx2 and up-regulated in Pax6 mutant telencephalon. Dev Dyn 231:614–620

Feng J, Bi C, Clark BS, Mady R, Shah P, Kohtz JD (2006) The Evf-2 noncoding RNA is transcribed from the Dlx-5/6 ultraconserved region and functions as a Dlx-2 transcriptional coactivator. Genes Dev 20:1470–1484

Garbe JC, Bendena WG, Alfano M, Pardue ML (1986) A *Drosophila* heat shock locus with a rapidly diverging sequence but a conserved structure. J Biol Chem 261:16889–16894

Geyer PK, Corces VG (1987) Separate regulatory elements are responsible for the complex pattern of tissue-specific and developmental transcription of the *yellow* locus in *Drosophila melanogaster*. Genes Dev 1:996–1004

Geyer PK, Spana C, Corces VG (1986) On the molecular mechanism of gypsy-induced mutations at the *yellow* locus of *Drosophila melanogaster*. EMBO J 5:2657–2662

Gibert JM, Simpson P (2003) Evolution of *cis*-regulation of the proneural genes. Int J Dev Biol 47:643–651

Gummalla M, Maeda RK, Castro Alvarez JJ, Gyurkovics H, Singari S, Edwards KA, Karch F, Bender W (2012) *abd-A* regulation by the *iab-8* noncoding RNA. PLoS Genet 8:e1002720

Guo M, Bier E, Jan LY, Jan YN (1995) *tramtrack* acts down-stream of *numb* to specify distinct daughter cell fates during asymmetric cell divisions in the *Drosophila* PNS. Neuron 14:913–925

Guttman M, Rinn JL (2012) Modular regulatory principles of large non-coding RNAs. Nature 482:339–346

Hardiman KE, Brewster R, Khan SM, Deo M, Bodmer R (2002) The *bereft* gene, a potential target of the neural selector gene *cut*, contributes to bristle morphogenesis. Genetics 161:231–247

Heo JB, Sung S (2011) Vernalization-mediated epigenetic silencing by a long intronic noncoding RNA. Science 331:76–79

Hirota K, Miyoshi T, Kugou K, Hoffman CS, Shibata T, Ohta K (2008) Stepwise chromatin remodelling by a cascade of transcription initiation of non-coding RNAs. Nature 456:130–134

Hyashizaki Y (2004) Mouse transcriptome: neutral evolution of 'non-coding' complementary DNAs. Nature 431:757

Inagaki S, Numata K, Kondo T, Tomita M, Yasuda K, Kanai A, Kageyama Y (2005) Identification and expression analysis of putative mRNA-like non-coding RNA in *Drosophila*. Genes Cells 10:1163–1173

Kapranov P, Cheng J, Dike S, Nix DA, Duttagupta R, Willingham AT, Stadler PF, Hertel J, Hackermüller J, Hofacker IL, Bell I, Cheung E, Drenkow J, Dumais E, Patel S, Helt G, Ganesh M, Ghosh S, Piccolboni A, Sementchenko V, Tammana H, Gingeras TR (2007) RNA maps reveal new RNA classes and a possible function for pervasive transcription. Science 316:1484–1488

Lakhotia SC (2003) The non coding developmentally active and stress inducible *hsrω* gene of *Drosophila melanogaster* integrates post-transcriptional processing of other nuclear transcripts. In: Barciszewski J, Erdmann VA (eds) Noncoding RNAs: molecular biology and molecular medicine. Kluwer Academic/Plenum, New York, pp 203–221

Lakhotia SC, Rajendra T, Prasanth KV (2001) Developmental regulation and complex organization of the promoter of the noncoding *hsrw* gene of *Drosophila melanogaster*. J Biosci 26:25–38

Lakhotia SC, Mallik M, Singh AK, Ray M (2012) The large noncoding *hsrω*-n transcripts are essential for thermotolerance and remobilization of hnRNPs, HP1 and RNA polymerase II during recovery from heat shock in *Drosophila*. Chromosoma 121:49–70

Lewis EB (1978) A gene complex controlling segmentation in *Drosophila*. Nature 276:565–570

Li M, Wen S, Guo X, Bai B, Gong Z, Liu X, Wang Y, Zhou Y, Chen X, Liu L, Chen R (2012) The novel long non-coding RNA *CRG* regulates *Drosophila* locomotor behavior. Nucleic Acids Res 40:11714–11727

Li M, Xu M, Wen S, Bai B, Chen R, Liu L (2014) One novel long noncoding RNA *nc10* in *Drosophila*. JGG 41:79–82

Lipshitz HD, Peattie DA, Hogness DS (1987) Novel transcripts from the ultrabithorax domain of the bithorax complex. Genes Dev 1:307–322

Martens JA, Laprade L, Winston F (2004) Intergenic transcription is required to repress the *Saccharomyces cerevisiae SER3* gene. Nature 429:571–574

Martianov I, Ramadass A, Serra Barros A, Chow N, Akoulitchev A (2007) Repression of the human dihydrofolate reductase gene by a non-coding interfering transcript. Nature 445:666–670

Martin JR, Ollo R (1996) A new *Drosophila* Ca^{2+}/calmodulin-dependent protein kinase (Caki) is localized in the central nervous system and implicated in walking speed. EMBO J 15:1865–1876

Mehler MF, Mattick JS (2006) Non-coding RNAs in the nervous system. J Physiol 575:333–341

Mehler MF, Mattick JS (2007) Noncoding RNAs and RNA editing in brain development, functional diversification, and neurological disease. Physiol Rev 87:799–823

Mercer TR, Dinger ME, Sunkin SM, Mehler MF, Mattick JS (2008) Specific expression of long noncoding RNAs in the mouse brain. Proc Natl Acad Sci USA 105:716–721

Modolell J, Campuzano S (1998) The achaete-scute complex as an integrating device. Int J Dev Biol 42:275–282

Muotri AR, Gage FH (2006) Generation of neuronal variability and complexity. Nature 441:1087–1093

Mutsuddi M, Lakhotia SC (1995) Spatial expression of the *hsr-omega* (93D) gene in different tissues of *Drosophila melanogaster* and identification of promoter elements controlling its developmental expression. Dev Genet 17:303–311

Nash WG, Yarkin RJ (1974) Genetic regulation and pattern formation: a study of the *yellow* locus in *Drosophila melanogaster*. Genet Res 24:19–26

Negre B, Simpson P (2009) Evolution of the achaete-scute complex in insects: convergent duplication of proneural genes. Trends Genet 25:147–152

Ng SY, Lin L, Soh BS, Stanton LW (2013) Long noncoding RNAs in development and disease of the central nervous system. Trends Genet 29:461–468

Pang KC, Frith MC, Mattick JS (2006) Rapid evolution of noncoding RNAs: lack of conservation does not mean lack of function. Trends Genet 22:1–5

Pease B, Borges AC, Bender W (2013) Noncoding RNAs of the ultrabithorax domain of the *Drosophila* bithorax complex. Genetics 195:1253–1264

Petruk S, Sedkov Y, Riley KM, Hodgson J, Schweisguth F, Hirose S, Jaynes JB, Brock HW, Mazo A (2006) Transcriptional elongation of non-coding *bxd* RNAs promoted by the trithorax TAC1 complex represses *Ubx* by a transcriptional interference mechanism. Cell 127:1209–1221

Ponjavic J, Ponting CP, Lunter G (2007) Functionality or transcriptional noise? Evidence for selection within long noncoding RNAs. Genome Res 17:556–565

Ponjavic J, Oliver PL, Lunter G, Ponting CP (2009) Genomic and transcriptional co-localization of protein-coding and long non-coding RNA pairs in the developing brain. PLoS Genet 5:e1000617

Qureshi IA, Mattick JS, Mehler MF (2010) Long non-coding RNAs in nervous system function and disease. Brain Res 1338:20–35

Rhyu MS, Jan LY, Jan YN (1994) Asymmetric distribution of numb protein during division of the sensory organ precursor cell confers distinct fates to daughter cells. Cell 76:477–491

Rinn JL, Chang HY (2012) Genome regulation by long noncoding RNAs. Annu Rev Biochem 81:145–166

Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, Goodnough LH, Helms JA, Farnham PJ, Segal E, Chang HY (2007) Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell 129:1311–1323

Sipos L, Kozma G, Molnár E, Bender W (2007) In situ dissection of a Polycomb response element in *Drosophila melanogaster*. Proc Natl Acad Sci USA. 104:12416–12421

Slawson JB, Kuklin EA, Ejima A, Mukherjee K, Ostrovsky L, Griffith LC (2011) Central regulation of locomotor behavior of *Drosophila melanogaster* depends on a CASK isoform containing CaMK-like and L27 domains. Genetics 187:171–184

Soshnev AA, Li X, Wehling MD, Geyer PK (2008) Context differences reveal insulator and activator functions of a Su (Hw) binding region. PLoS Genet 4:e1000159

Soshnev AA, Ishimoto H, McAllister BF, Li X, Wehling MD, Kitamoto T, Geyer PK (2011) A conserved long noncoding RNA affects sleep behavior in *Drosophila*. Genetics 189:455–468

Stark A, Bushati N, Jan CH, Kheradpour P, Hodges E, Brennecke J, Bartel DP, Cohen SM, Kellis M (2008) A single Hox locus in *Drosophila* produces functional microRNAs from opposite DNA strands. Genes Dev 22:8–13

Tyler DM, Okamura K, Chung WJ, Hagen JW, Berezikov E, Hannon GJ, Lai EC (2008) Functionally distinct regulatory RNAs generated by bidirectional transcription and processing of microRNA loci. Genes Dev 22:26–36

Uemura T, Shepherd S, Ackerman L, Jan LY, Jan YN (1989) *numb*, a gene required in determination of cell fate during sensory organ formation in *Drosophila* embryos. Cell 58:349–360

Willingham AT, Orth AP, Batalov S, Peters EC, Wen BG, Aza-Blanc P, Hogenesch JB, Schultz PG (2005) A strategy for probing the function of noncoding RNAs finds a repressor of NFAT. Science 309:1570–1573

Young RS, Marques AC, Tibbit C, Haerty W, Bassett AR, Liu JL, Ponting CP (2012) Identification and properties of 1,119 candidate lincRNA loci in the *Drosophila melanogaster* genome. Genome Biol Evol 4:427–442

Zhao J, Sun BK, Erwin JA, Song JJ, Lee JT (2008) Polycomb proteins targeted by a short repeat RNA to the mouse X chromosome. Science 322:750–756